Amendments to the Specification:

Please replace the paragraph at page 6, lines 12-27, with the following amended paragraph:

The present invention further encompasses methods of treatment or prevention of an eye disorder, or a complication of an eye disorder, comprising providing a subject with a first composition comprising at least one TPCD, and a second composition comprising at least one TPCD. In one embodiment of this aspect of the invention, a first composition comprising at least one TPCD and a second composition comprising at least one TPCD are provided to a subject by contacting a vitreous and/or an aqueous humor. In another embodiment of this aspect of the invention, the TPCDs of the first composition comprising at least one TPCD and the second composition comprising at least one TPCD are the same TPCD. In yet another embodiment of this aspect of the invention, the TPCDs of the first composition comprising at least one TPCD and the second composition comprising at least one TPCD are different TPCDs. In a further embodiment of this aspect of the invention, the first composition comprising at least one TPCD and the second composition comprising at least one TPCD are administered to a subject at substantially the same time. In yet another embodiment, the first composition comprising at least one TPCD and the second ~~compositions~~ composition comprising at least one TPCD are administered to a subject at separate times.

Please replace the paragraph at page 7, lines 11-19, with the following amended paragraph:

The methods of the invention can be used to treat or prevent an eye disorder, or a complication of an eye disorder, of a subject by effecting one or more outcomes including, but not limited to, reducing the viscosity of the vitreous, liquefying the vitreous, inducing posterior vitreous detachment, clearing or reducing hemorrhagic blood from the vitreous and/or aqueous humor, clearing or reducing intraocular foreign substances from the vitreous and/or aqueous humor, clearing or reducing materials toxic to the retina, increasing diffusion of an agent or a composition administered to the ~~vitreousand/or~~ vitreous and/or aqueous humor, reducing extraretinal neovascularization and any combinations thereof.

Please replace the paragraph at page 8, lines 4-5, with the following amended paragraph:

FIG. 2 provides the DNA (SEQ ID NO:3) and amino acid sequence ~~(SEQ ID NO:4)of~~ (SEQ ID NO:4) of human microplasminogen.

Please replace the paragraph bridging pages 9-10, with the following amended paragraph:

~~FIG.10~~ FIG. 10 presents the results of confocal laser scanning microscopy with probes to glial fibrillic acidic protein (Panels A and B, green) and vimentin (Panels C and D, red). There is no difference in the staining of GFAP and vimentin between microplasmin-treated eyes (Panels A and C) and control eyes (Panels B and D). Double-label immunohistochemistry with probes to synaptophysin (green) and neurofilament (red) also shows no difference between microplasmin-treated eyes (Panel E) and a control eyes (Panel F). Magnification for Panels [[A,B]] A, B and C was 400x; magnification for Panel D was 250X; and magnification for Panels E and F was 160x.

Please replace the paragraph at page 15, lines 10-18, with the following amended paragraph:

Accordingly, the present invention provides, as a first aspect, a method of treating or preventing a disorder, or a complication of a disorder, of the eye of a subject comprising contacting the vitreous and/or aqueous humor with an effective amount of a composition comprising a TPCD. In one embodiment, a TPCD has a molecular weight less than about 40,000 daltons. In another embodiment, a TPCD has a molecular weight of about 26,500 daltons in reduced form or about 29,000 daltons in non-reduced form. In yet another embodiment a TPCD has a molecular weight of between about 20,000 and 30,000 daltons. In a further embodiment, a TPCD has a molecular ~~wight~~ weight of less than about 20,000 daltons.

Please replace the paragraph bridging pages 15-16, with the following amended paragraph:

In a third aspect, the present invention provides a method of treating or preventing a disorder, or a complication of a disorder, of the eye of a subject comprising contacting the vitreous and/or aqueous humor with an effective amount of a first composition comprising at least one TPCD and an effective amount of a second composition comprising at least one TPCD. In one embodiment of this aspect of the invention, the first composition comprising at least one TPCD and the second composition comprising at least one TPCD can comprise the same ~~TPCD~~ ~~In~~ TPCD. In another embodiment of this aspect of the invention, the first composition comprising at least one TPCD and the second composition comprising at least one TPCD can comprise different TPCDs. In a further embodiment of this aspect of the invention, the first and second compositions may be administered to a subject at substantially the same time or at different times.

Please replace the paragraph bridging pages 19-21, with the following amended paragraph:

TPCD includes, but is not limited to, miniplasmin, recombinant miniplasmin, stabilized miniplasmin, stabilized, recombinant miniplasmin, variants of miniplasmin, microplasmin, recombinant microplasmin, stabilized microplasmin, stabilized, recombinant microplasmin, and variants of microplasmin. Variants of microplasmin and miniplasmin include shorter forms of microplasmin and miniplasmin that can be produced by amino acid deletions from these proteins. All variants of microplasmin and miniplasmin are expected to have serine protease catalytic activity, even if they do not possess the same level of catalytic activity as microplasmin and miniplasmin, respectively. Thus, all variants of microplasmin and miniplasmin are required to contain amino acids 603-741 of SEQ ID NO:10, which contains the catalytic triad of the plasmin serine protease domain namely, His_{603}, Asp_{646} and Ser_{741} of human plasminogen. In one ~~embodiment~~ embodiment, a variant of miniplasmin includes proteins containing one or more amino acid deletions in amino acids 444-791 of human plasminogen, wherein the resulting protein possesses serine protease catalytic activity. In another embodiment, a variant of

microplasmin includes proteins containing one or more deletions in amino acids 543-791 of human plasminogen, wherein the resulting protein possesses serine protease catalytic activity. In yet another embodiment, a variant of microplasmin includes proteins containing one or more deletions in amino acids 562-791 of human plasminogen, wherein the resulting protein possesses serine protease catalytic activity. The deletions can be at the N-terminus, ~~C terminus~~ C-terminus, or at an internal location of amino acids 444-791, 543-791 and 562-791 of SEQ ID NO:10, respectively; however, all variants of microplasmin and miniplasmin are required to contain amino acids 603-741 of SEQ ID NO:10. Variants of microplasmin and miniplasmin also include, but are not limited to, amino acid insertions and/or substitutions in these proteins. It is envisioned that amino acid substitutions made in microplasmin or miniplasmin are preferably conservative substitutions. Any variant of microplasmin and miniplasmin or any other TPCD can be prepared by recombinant methods and activated to the active plasmin form with a plasminogen activator. Alternatively, variants of microplasmin and miniplasmin or any other TPCD can be prepared by any other means well known in the art such as, but not limited to, digestion of human plasminogen with elastase or partial reduction and alkylation of plasmin, microplasmin or miniplasmin. These variants of microplasmin, miniplasmin, or for that ~~matter~~ matter, any TPCD, can be assayed for serine protease catalytic activity using the chromogenic substrate S2403 or any other chromogenic substrate. In addition, the variants of microplasmin, miniplasmin or any other TPCD can be tested for their ability to induce PVD and/or effect vitreous liquefaction by injecting different doses of the variant in any balanced saline solution into porcine, feline or post-mortem human eyes. If a TPCD can induce PVD and/or effect vitreous liquefaction in any of these eyes, that TPCD is considered to be useful for treating eye disorders of mammals. Preferably, the TPCD does not result in toxicity to the injected eye. Non-limiting examples of variants of microplasmin are provided in Table 1.

Please replace the paragraph bridging pages 31-32, with the following amended paragraph:

Microplasmin can be prepared by the autolytic reaction of plasmin and plasminogen in high alkaline solution having a pH ranging from about 9.5 to 11.5, as described in U.S. Patent

No. 4,774,087. Alternatively, microplasmin and miniplasmin can be prepared by recombinant methods as described in PCT application WO 02/50290. Briefly, DNA encoding miniplasminogen and microplasminogen are independently cloned into a yeast expression vector ([[*for*]] *e.g.*, pPICZα A secretion vector from Invitrogen Corporation) that can be used to express these proteins in methylotropic yeasts (*e.g., Hansenula, Pichia, Candida,* and *Torulopsis).* Yeast clones that produce proteins with the highest miniplasmin and microplasmin activity are selected for large-scale production. These clones can be grown at any scale, but typically at about a 20 liter to about a 500 liter scale. The secreted miniplasminogen or microplasminogen are purified in a three-step process comprising cation exchange expanded bed chromatography, hydrophobic chromatography, and affinity chromatography. The purified microplasminogen and miniplasminogen obtained by this process are activated to their active forms using a molar ratio of a plasminogen activator (*e.g.*, urokinase, streptokinase, staphylokinase, the SY162 staphylokinase variant, etc.). It should be noted that the recombinant process for producing miniplasmin and microplasmin can be extended to produce any TPCD. An advantage of using a recombinant TPCD compared to autologous plasmin enzyme is that the recombinant proteins can be prepared from large production batches resulting in enzymes of uniform activity. Because these proteins are of uniform activity, standardized protocols can be implemented. A further advantage is that these proteins could be readily available without the delay and other attendant problems associated with the isolation and purification of plasmin from each patient.

Please replace the paragraph at page 32, lines 11-26, with the following amended paragraph:

Stabilization is a method of protecting a protein from degradation and/or inactivation through the use of one or more stabilizing agents ([[*for*]] *e.g.*, by contacting a TPCD with a stabilizing agent, or purifying a TPCD in the presence of a stabilizing agent). Stabilizing agents include without limitation, tranexamic acid, hexanoic acid, lysine, serine, threonine, methionine, glutamine, alanine, glycine, isoleucine, valine, alanine aspartic acid, polyhydric alcohol, pharmaceutically acceptable carbohydrates, glucosamine, thiamine, niacinamide, any acidic buffer comprising citric acid, acetic acid, hydrochloric acid, carboxylic acid, lactic acid, malic

acid, tartaric acid, or benzoic acid, and salts such as sodium chloride, potassium chloride, magnesium chloride, calcium chloride, and any derivatives or combinations thereof. One advantage of using stabilized, recombinantly produced TPCD compared to autologous plasmin enzyme is that these proteins are more stable than autologous plasmin enzyme, which is obtained by collecting blood and, purifying, preparing and storing plasmin enzyme on a patient-by-patient basis. Unlike autologous plasmin enzyme, which needs to be used very soon after its preparation, stabilized, recombinant TPCD can be used even after a significant period of time from the time of purification.

Please replace the paragraph bridging pages 34-35, with the following amended paragraph:

The present invention also contemplates the use of compositions comprising more than one TPCD. Accordingly, in one aspect of the invention, the vitreous and/or aqueous humor is contacted with a composition comprising a first TPCD and a second TPCD. In one particular embodiment of this aspect of the invention, the first and second TPCD are selected from the group consisting of miniplasmin, recombinant miniplasmin, stabilized miniplasmin, stabilized, recombinant miniplasmin, variants of miniplasmin, microplasmin, recombinant microplasmin, stabilized microplasmin, stabilized, recombinant microplasmin, variants of microplasmin, and any combinations thereof. In another aspect of the invention, the vitreous and/or aqueous humor is contacted with a first composition comprising at least one TPCD and with a second composition comprising at least one TPCD. The TPCD can be the same or different proteins and can be administered at substantially the same time or at different times. Additionally, a TPCD can also be administered as a composition further comprising at least one second agent. Furthermore, the vitreous and/or aqueous humor may be contacted with a composition comprising at least one TPCD followed by a composition comprising at least one second agent or *vice versa*. This may be necessary where the time required for each of these compositions is different, i.e., where one ~~compositions~~ composition needs more time to act compared to the other. A second agent is any protein (but not a TPCD), chemical or other substance that is useful in treating or preventing eye disorders, or complications of an eye disorder. Such second

agents are described in U.S. Patent Nos.: 4,820,516; 5,292,509; 5,866,120; 6,051,698; 6,462,071; 6,596,725; and 6,610,292. Non-limiting examples of second agents usable with the present invention include glycosaminoglycanase enzymes such as hyaluronidases, chondroitinase ABC, chondroitinase AC, chondroitinase B, chondroitin 4-sulfatase, chondroitin 6-sulfatase and B-glucuronidase; collagenase enzymes; dispase; RGD containing peptides such as RGD, GRGDS (SEQ ID NO:11), GRGDTP (SEQ ID NO:12), Echistatin and Falvoridin; anti-integrin antibody; P2Y receptor antagonists; urea, hydroxyurea, thiourea and anti-angiogenic agents such as, but not limited to, vascular endothelial growth factor (VEGF) inhibitors (*e.g.*, anti-VEGF antibodies, VEGF aptamers, soluble VEGF receptors, etc.) and placental growth factor (PlGF) inhibitors (*e.g.*, anti-PlGF antibodies, PlGF aptamers, soluble VEGF receptors, etc.). Most of these second agents are themselves capable of promoting vitreous liquefaction and/or inducing posterior vitreous detachment. Anti-angiogenic second agents could be useful in preventing neo-vascularization in the eye. Expression of VEGF and/or PlGF from an hypoxic retina are thought to result in the development of extraretinal neovascularization. Thus, inhibiting VEGF and/or PlGF would be an effective way to prevent neovascularization.

Please replace the paragraph bridging pages 37-38, with the following amended paragraph:

Several ophthalmological disorders including diabetic retinopathy and trauma result in the rupture or leakage of retinal blood vessels with resultant bleeding into the vitreous (*i.e.*, vitreous hemorrhage). Vitreous hemorrhage typically manifests as clouding or opacification of the vitreous and is sometimes, but not always, accompanied by tearing or detachment of the retina. In cases where the vitreous hemorrhage is accompanied by a retinal tear or detachment, it is important that such retinal tear or detachment be promptly diagnosed and surgically repaired. Failure to promptly diagnose and repair the retinal tear or detachment may allow photoreceptor cells of the retina, in the region of the tear or detachment, to become necrotic. Necrosis of the photoreceptor cells of the retina may result in loss of vision. Furthermore, allowing the retinal detachment to remain unrepaired for such extended period of time may result in further vitreous hemorrhage and/or the formation of fibrous tissue at the site of the

~~hemorrhage. Fibrous~~ hemorrhage. Fibrous tissue may result in the formation of an undesirable permanent fibrous attachment between the vitreous body and the retina. In the absence of any treatment, hemorrhagic clouding of the vitreous can take between 6-12 months or longer to clear sufficiently to allow trans-vitreal viewing of the retina. In such cases, where a physician would need to repair any part of the retinal surface, or where a physician would need to view the retinal surface of a patient that is prevented by an opaque or cloudy vitreous, a microsurgical procedure known as vitrectomy may need to be performed. This procedure involves removal of all or a portion of the vitreous with a microsurgical cutter and the replacement of the vitreous with a clear liquid or other substance that allows the ocular cavity to maintain its shape. Standard vitrectomy surgical procedures are well known to those of ordinary skill in the art. In one embodiment, the present invention contemplates contacting the vitreous with a composition comprising at least one TPCD as an adjunct to vitrectomy. In other embodiments, the vitreous is contacted with the composition comprising at least one TPCD in the absence of performing a vitrectomy.

Please replace the paragraph bridging pages 38-39, with the following amended paragraph:

The pPICZα A secretion vector purchased from Invitrogen Corporation (Carlsbad, California) was used to direct expression and secretion of recombinant human microplasminogen and miniplasminogen in *Pichia pastoris*. Notable features of this vector include: (i) a 942 bp fragment containing the alcohol oxidase 1 (*AOX1*) promoter that allows methanol-inducible, high level expression of recombinant protein in *Pichia*, as well as targeted plasmid integration to the *AOX1* chromosomal locus; (ii) the native transcription termination and polyadenylation signal from the *AOX1* gene; (iii) an expression cassette conferring zeocin resistance to *Escherichia coli* and *Pichia pastoris*; (iv) a ColE1 origin of replication for propagation and maintenance of the plasmid in *Escherichia coli*; (v) a c-myc epitope and a polyhistidine (6X His) tag (SEQ ID NO:13), which can be used for protein detection and purification; and (vi) unique restriction sites (for example, Sac I, Pme I, BstXI) that permit linearization of the vector at the *AOX1* locus for efficient integration into the *Pichia* genome.

Please replace the paragraph on page 39, lines 14-17, with the following amended paragraph:

1. the preliminary cleavage of the signal sequence by the *KEX2* gene product occurring between arginine and glutamine in the sequence Glu-Lys-Arg * Glu-Ala-Glu-Ala (SEQ ID NO:14), where * is the site of cleavage. However, the Glu-Ala repeats are not always necessary for cleavage by Kex2.

Please replace the paragraph on page 40, lines 16-23, with the following amended paragraph:

The LY-MPLG1 primer had an annealing region corresponding to residues 543-548 (Ala-Pro-Ser-Phe-Asp-Cys) of human plasminogen ~~(Ala Pro Ser Phe Asp Cys)~~ (SEQ ID NO:10) preceded by a non-annealing extension which included the last four residues of the α factor mating signal (Leu-Glu-Lys Arg) (SEQ ID NO:15). In this extension, the Leu-Glu codons determine the Xho I restriction site (underlined) allowing the cloning of the gene of interest flush with the Kex2 cleavage site. The LY-MPLG2 primer had an annealing region corresponding to the last seven residues of plasminogen, followed by a TAA stop-codon and a non-annealing region comprising a Xba I recognition sequence (underlined).

Please replace the paragraph on page 42, lines 1-6, with the following amended paragraph:

The LY-MINPLG1 primer has an annealing region corresponding to residues 444-452 (Ala-Pro-Pro-Pro-Val-Val-Leu-Leu-Pro) of plasminogen ~~(Ala Pro Pro Pro Val Val Leu Leu Pro)~~ (SEQ ID NO:10) preceded by a non-annealing extension which included the last four residues of the factor mating signal (Leu-Glu-Lys-Arg) (SEQ ID NO:15). In this extension, the Leu-Glu codons determine the Xho I restriction site (underlined) allowing the cloning of the gene of interest flush with the Kex2 cleavage site.

Please replace the paragraph on page 42, lines 7-10, with the following amended paragraph:

The LY-MINPLG2 primer has an annealing region corresponding to the residues 596-604 (Glu-Trp-Val-Leu-Thr-Ala-Ala-His-Cys) of human plasminogen ~~(Glu-Trp-Val-Leu-Thr-Ala-Ala-His-Cys)~~ (SEQ ID NO:10). This annealing region of the catalytic domain, also present in the microplasminogen expression vector, comprises a unique Pst I recognition sequence (underlined).

Please replace the paragraph bridging pages 63-64, with the following amended paragraph:

A new compact DLS fiber-optic probe (U.S. Patent No. 5,973,779) was fabricated for the vitreous studies described herein. It comprises of a pair of 0.25 pitch Selfoc GRIN lenses, and it has a penetration depth of ~16 mm and a scattering angle of 160°. A fiber optic probe comprising two monomode optical fibers and two GRIN lenses provides a compact and remote means of studying the dynamic characteristics of the macromolecules in the eye. Two monomode optical fibers, each housed in a stainless steel ferrule, are mounted into a separate stainless steel housing. An air gap is intentionally left between the fiber housing and the lens housing in order to produce a tightly focused spot in the scattering volume. The two optical fibers in their housings are aligned and fixed into position off-axis with the micro lens. The two housings are placed inside a third (outer) housing made of stainless steel, and the back end of the housing is covered with a ~~heat-shrink~~ heat-shrink tubing. The two free ends of the optical fibers were terminated with FC/PC-type male connectors for easy mating with the laser and photo-detector module.

Please replace the paragraph bridging pages 68-69, with the following amended paragraph:

In this ~~experiment~~ experiment, DLS was used to non-invasively assess molecular structure in vitreous by measuring particle sizes, scattering intensity, and polydispersity. The results showed that there are similar DLS profiles in various locations within whole vitreous.

The most pronounced effects of microplasmin were upon whole vitreous incubated at ~~37 degrees C~~ 37°C for 30 min, especially at higher doses. There was a substantial diminution in normalized average particle size and a statistically significant dose-response relation was established. This suggests that μPli would be useful as an adjunct for vitreo-retinal surgery, since a 30 minute time frame is reasonable for a drug effect that does not interfere with current surgical practices. In conjunction with the data in the previous Examples suggesting that μPli induces dehiscence at the vitreo-retinal interface, this drug appears to achieve the two desired components for pharmacologic vitreolysis: posterior vitreous detachment and a breakdown in vitreous macromolecules with consequent increases in vitreous diffusion coefficients and ultimately liquefaction.

Please replace the Abstract at page 81, lines 2-11, with the following replacement Abstract:

~~A method~~ Methods of treating or preventing a disorder, or a complication of a disorder, of an eye of a ~~subject~~ subject, comprising contacting a vitreous and/or aqueous humor with a composition comprising a truncated form of plasmin comprising a catalytic domain of plasmin (TPCD) are disclosed. TPCDs include, but are not limited to, miniplasmin, microplasmin and derivatives and variants thereof. The methods of the invention can be used to reduce the viscosity of the vitreous, liquefy the vitreous, induce posterior vitreous detachment, reduce hemorrhagic blood from the eye, clear or reduce materials toxic to the eye, clear or reduce intraocular foreign substances from the eye, increase diffusion of a composition administered to an eye, reduce extraretinal neovascularization and any combinations thereof. The method can be used in the absence of, or as an adjunct to, vitrectomy.

Please insert the attached Sequence Listing after the last page of the application.